ORIGINAL ARTICLE

Lack of association of sodium-lithium counter transport with microalbuminuria in young diabetics of Bangladesh

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Background: Raised sodium-lithium counter transport activity (SLCT) in red blood cells (RBC) is claimed to be an early marker of nephropathy in type 1 and type 2 diabetic patients. This study aimed to investigate whether SLCT changes parallel with early nephropathy in young diabetics of Bangladesh. **Materials and Methods:** Thirty-five newly diagnosed, untreated, young and normotensive Bangladeshi diabetics without ketoacidosis, family history of hypertension and renal disease, and 19 age and body mass index (BMI) matched healthy controls without family history of hypertension and diabetes were studied to investigate whether their blood glucose, C-peptide, lipids and microalbuminuria were related with abnormality of SLCT activity. **Results:** Diabetic subjects had extremely variable degree of serum glucose (mmol/L) (mean ± SD) (fasting 16.6 5.8; postprandial 29.7 ± 7.5) and C-peptide (pmol/L) [median (range) (fasting 0.289 (0.028–0.994); postprandial 0.379 (0.053–2.424)], significantly higher ($P < 0.05$) urinary albumin creatinine ratio (ACR) and no difference in SLCT when compared to healthy controls. But on subgrouping of diabetic patients according to fasting C-peptide value 0.16 pmol/L as a cut-off point, subjects with low C-peptide showed higher ($P < 0.01$) ACR than the controls and higher ($P < 0.05$) SLCT than moderate C-peptide. Microalbuminuria with ACR > 2.6 (mean + 2SD of control) was found in seven (20%) diabetics with no difference of SLCT between normoalbuminuric and microalbuminuric diabetics. **Conclusions:** SLCT activity in RBC is not a predictor of nephropathy in young diabetics of Bangladesh and the development of nephropathy is mainly related to glycemic and insulin status. SLCT may be elevated by marked hypoinsulinemia even without hypertension or family history of hypertension.

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Introduction

Nephropathy is a common microvascular complication of both type 1 and type 2 diabetes mellitus.[1] Microalbuminuria[2,3] and microtransferrinuria[4,5] are the earliest detectable clinically useful markers of diabetic nephropathy. These abnormalities develop after the increase in glomerular filtration rate, the hallmark of early renal hemodynamic changes.[6] Detection of a marker preceding the earliest renal abnormalities, which may predict future nephropathy, and timely intervention to reduce morbidity and mortality are the highly desirable goals in diabetology as well as in nephrology.

Elevation of sodium-lithium counter transport (SLCT) activity in red blood cells (RBC), a genetic marker of essential hypertension,[7,8] has also been proposed as a marker of nephropathy in type 1[9-13] and type 2[14,15] diabetic subjects. However, in most of these studies the raised SLCT was claimed to be due to susceptibility to hypertension. Metabolic abnormalities related to diabetes *per se*[16-18] are also claimed to raise the SLCT. Elucidation of this distinction is important to decide whether

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b) 4th Diabetes and Endocrine Conference of Diabetic Association of Bangladesh and Bangladesh Endocrine Society, 12–13 December, 1998.

SLCT can be used as a clinically useful early marker of nephropathy. Much of the confusion in this area is due to difficulties in designing studies, particularly in human subjects, where confounding variables like hypertension or family history of hypertension,[7,8] hyperglycemia,[19] hyperlipidemia,[20] hyperinsulinemia and insulin resistance[21-23] can be studied with minimum interdependence.

Like some other countries in the tropics, Bangladesh has a group of young (age < 30 years) diabetic patients who were grouped and studied as a separate entity – malnutrition related diabetes mellitus (MRDM) with subgroups fibrocalculus pancreatic diabetes (FCPD) and protein deficient diabetes mellitus (PDDM) as they do not resemble typically type 1 and type 2 diabetes, and have peculiar clinical and metabolic features like low body mass index (BMI), normotension, minimum dyslipidemia, considerable degree of hyperglycemia and hypoinsulinemia and no ketoacidosis even in an untreated state.[24-27]

These young diabetics are susceptible to nephropathy as evidenced by early renal hemodynamic [(substantial rise of glomerular filtration rate (GFR)] and microvascular (microalbuminuria/microtransferrinuria) changes observed in FCPD patients in Bangladesh[28] and microalbuminuria observed in MRDM subjects with clinically phasic insulin dependence diabetes mellitus (PIDDM) in Jamaica.[29] But there had been no study on SLCT and its relation with the development of nephropathy in these young diabetics of tropics. So, this study was undertaken to verify whether the SLCT could be the independent predictor of nephropathy in these patients without a family history of hypertension and to explore its relationship with glycemic and insulin status, lipid levels and microalbuminuria.

Materials and Methods

Thirty-five newly diagnosed young (age 13–29 years) diabetics (16 males and 19 females) consecutively attending the out patient department of BIRDEM from July 1997 to Dec 1997 were included. Diabetics with ketosis, chronic illness, urinary tract infection, non-diabetic renal disease, overt proteinuria (albustix positive) due to any disease, pregnancy, already on anti-diabetic treatment and parental history of essential hypertension were excluded from the study. Nineteen age-matched (age 15–29 years) healthy individuals (11 males and 8 females) without parental history of hypertension and diabetes up to second generation were selected as controls. After selection of the subjects, an appointment was given and they were asked to fast overnight. On the date of appointment, height (cm) and weight (kg) were measured with light clothing and without shoes for calculation of BMI (weight in kg divided by height in m^2). Blood pressure was measured by mercury sphygnomanometer with standard sized cuff (12 × 3 inch) after 10–15 minutes of rest, and the mean of two readings was taken.

Urine sample was collected in a clean test tube and centrifuged at a rate of 3000 rpm for 10 minutes. Then 1.5 mL of clear urine sample was transferred to a microcentrifuge tube and preserved at –70°C for future determination of urinary creatinine by alkaline picrate method and urinary albumin excretion (microalbuminuria) by immunoturbidimetric method (Ames, Miles Laboratories, Elkhart, Ind, USA). Albumin creatinine ratio (ACR) was calculated and expressed as mg of albumin/mmol of creatinine.

Fasting and postprandial (2 hours after taking 75 g of glucose) blood samples were drawn from the anti-cubital vein. Blood sample was allowed to clot and was centrifuged for 10 minutes at a rate of 3000 rpm. Serum separated was aliquoted and preserved in the freezer for future estimation of glucose (fasting and postprandial) by glucose-oxidase method, C-peptide (fasting and postprandial) by enzyme-linked immunosorbent assay (ELISA) technique, fasting total cholesterol, triglyceride (TG) and high density lipoprotein (HDL) by enzymatic method.

SLCT activity in RBC was determined according to the method described by Canessa *et al*.[7] Eight milliliters of fasting blood was taken in a heparin (10 µL/mL of blood) containing glass test tube. The test tube was sealed with parafilm and blood was mixed thoroughly by gentle inversion. The test tube was kept in ice until the experiment to determine SLCT was started. The heparinized blood was centrifuged at 3000 rpm at 4°C within 2 hours of collection. The plasma and buffy coat were discarded. Four different solutions were prepared everyday on the day of experiment either from previously prepared stock solutions of each ingredient or by weighing them on the same day of the experiment. The packed cells were washed three times with ice-cold "washing solution" containing $MgCl_2$ 75 mmol/L, sucrose 85 mmol/L, glucose 10 mmol/L, tris 10 mmol/L and MOPS (stands for (3-N-Morpholino propensulfonic acid (Mole weight 209.3). Purchased

from SIGMA, Sigma Chemical Co, 14508 St) 10 mmol/L with pH 7.4 and centrifuged at a rate of 3000 rpm at 4°C for 2 minutes. Then 1.5 mL of packed RBCs was added to 10 mL of "lithium containing medium" with lithium chloride 150 mmol/L, glucose 10 mmol/L, tris 10 mmol/L and MOPS 10 mmol/L at pH 7.4 in a culture flask in duplicate and incubated in a shaking water bath at 37°C at a rate of 50 rpm for 3 hours to load the RBCs with lithium. After incubation, the RBCs was transferred to test tubes and centrifuged at 4°C at 3000 rpm for 5 minutes. The packed cells were then washed four times with ice-cold washing solution by centrifuging at 4°C for 2 minutes at a rate of 3000 rpm. After completing the fourth wash, the packed cells were transferred to a fresh test tube and equal volume of ice-cold washing solution was added to it to make 50% suspension of RBCs. One milliliter of 50% RBC suspension was again incubated in fresh petridish containing 8 mL of Na-free medium with $MgCl_2$ 75 mmol/L, sucrose 85 mmol/L, tris 10 mmol/L and MOPS 10 mmol/L at pH 7.4 and Na-containing medium NaCl 150 mmol/L, ouabain 0.1 mmol/L, glucose 10 mmol/L, tris 10 mmol/L and MOPS 10 mmol/L with pH 7.4 in shaking water bath at a rate of 50 rpm at 37°C. From each petridish, 2 mL of medium was withdrawn at 15 and 30 minutes of incubation and transferred to 3-mL polysteron test tubes. The test tubes were immersed immediately into ice-cold water in order to stop the activity in the cells. Samples withdrawn at 15 and 30 minutes were centrifuged at 3000 rpm for 10 minutes at room temperature and dispensed in properly labeled microcentrifuge tubes and preserved in a freezer at –70°C for future determination of lithium. Then 500 µL of 50% RBC suspension was taken in microcentrifuge tubes and 200 µL of 1% triton-X100 was added to it. The cell suspension was vortexed properly to ensure lysis of all cells. The whole cell suspension was preserved in freezer at –70°C for future determination of total RBC protein content by enzymatic-colorimetric method by a BIO-RAD DC Protein Kit (BIO-RAD Detergent compatible protein assay kit), Bio-Rad Laboratories, Inc. 1000 Alfred Nobel Drive Hercules California, 94547 USA Lithium in the supernatant sample was determined by atomic absorption spectrophotometry.

The lithium transports out of the cells through sodium channels by diffusion in both media, and in addition, through SLCT pathway in sodium containing medium. So, the amount of lithium detected in the sodium free media was subtracted from the lithium detected in the sodium containing media both at 15 and 30 minutes, individually. The data obtained were multiplied by 4 and 2, respectively, and the mean of two was calculated to get the amount of lithium transported out through the SLCT in 1 hour. The activity was then expressed as µmol of lithium/g of RBC protein/hour.

SPSS windows package was used for the analysis of data. All parametric variables were expressed as mean ± SD unless otherwise stated. C-peptide, ACR and SLCT were expressed as median (range). The comparison between the groups was made either by unpaired t-test, Mann-Whitney U test or Duncan test for analysis of variance (ANOVA), and *P* values below 0.05 were considered significant.

Results

As shown in Table 1, diabetic subjects had extremely high serum glucose (fasting/postprandial) and low postprandial C-peptide, significantly lower systolic (SBP) and mean arterial blood pressure (MAP), significantly high TG and ACR and no difference of SLCT [Figure 1] when compared to the healthy subjects.

Previously young diabetics of Bangladesh were classified and studied as MRDM (with subgroups FCPD and PDDM) and noninsulin dependent diabetes of young (NIDDY).[24,26] The group MRDM was later omitted by the expert committee on the diagnosis and classification of diabetes mellitus[30] with division of diabetes mellitus into two broad groups, type 1 and type 2. So in this study diabetic subjects were classified according to insulin secreting capacity taking the fasting C-peptide (FCP) value <0.16 pmol/L as a cut-off point (the assumed cut-off point to differentiate type 1 and type 2 diabetes) into subjects with low FCP and moderate FCP.

Out of 35 diabetics, 14 subjects had low FCP resembling type 1 diabetics and 21 subjects had moderate FCP resembling type 2 diabetics. Comparison of these diabetics and healthy subjects is shown in Table 2. Diabetics with moderate FCP and healthy subjects had similar BMI, diastolic blood pressure (DBP) and MAP, whereas diabetics with low FCP showed significantly lower BMI, SBP, DBP and MAP than other subjects.

Diabetics with low FCP had significantly higher glucose level (fasting and postprandial) and significantly lower total cholesterol (T-chol) and low density lipoprotein cholesterol (LDL-chol) than diabetics with moderate FCP and had no difference in lipid levels when compared to healthy subjects, whereas diabetics with moderate FCP

showed significantly higher TG, T-chol and LDL than healthy subjects.

Comparison of ACR between diabetics showed no statistical difference. But diabetics with low FCP showed significantly higher ($P < 0.01$) ACR than control subjects. SLCT of both the diabetics were similar to control subjects. Comparison between the diabetics revealed significantly higher ($P < 0.05$) SLCT in diabetics with low FCP [Figure 2]. Diabetics were also studied after subgrouping according to ACR value 2.60 (mean ± 2SD of healthy subjects) as the cut-off point to differentiate microalbuminuric and normoalbuminuric diabetics. Twenty-eight diabetic subjects had normoalbuminuria with ACR < 2.60 and 7 (20%) diabetic subjects had microalbuminuria with ACR > 2.60. Out of seven microalbuminuric subjects, three were diabetics with low FCP and four were diabetics with moderate FCP. The diabetic subjects with microalbuminuria and normoalbuminuria showed no differences in age, BMI, SBP, MAP, DBP, fasting and postprandial sugar and C-peptide, T-chol, LDL-chol, HDL-chol, TG and SLCT [Figure 3].

Table 3 shows the correlation-coefficient study of different parameters in diabetic subjects. BMI and MAP showed positive correlation with C-peptide (fasting and postprandial) and negative correlation with glucose (fasting and postprandial). Serum TG, T-chol and LDL-chol also showed positive correlation with fasting and postprandial C-peptide. ACR of diabetics showed negative correlation with fasting and postprandial C-peptide and positive correlation with postprandial glucose.

A weak positive correlation between SLCT and postprandial glucose ($r = 0.37$, $P < 0.05$), weak negative correlation between SLCT and fasting C-peptide ($r = -0.41$, $P < 0.05$) and no correlation between SLCT and ACR was also observed in diabetic subjects.

Discussion

Marked hyperglycemia and hypoinsulinemia with significant difference between low FCP and moderate FCP subjects observed in newly diagnosed non-ketotic young diabetics of this study resembled that of the previous studies on MRDM and NIDDY.[24-27] But low

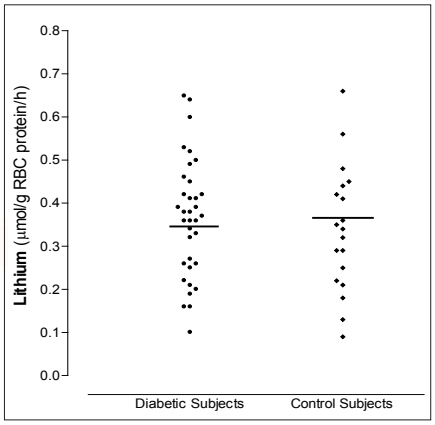

Figure 1: SLCT of diabetic subjects and healthy controls (n = 35) (n = 19) (n = 19) (n = 21) (n = 14)

Table 1: Clinical and biochemical characteristics, ACR and SLCT of the study populations

Groups	Diabetic subjects (n = 35)	Control subjects (n = 14)	*P* value
Age (years)[1]	22 ± 5	23 ± 4	<0.7
BMI (kg/m^2)[1]	18 ± 4	19.8 ± 2.8	0.09
SBP (mm Hg)[1]	94 ± 11	110 ± 12	0.001
DBP (mm Hg)[1]	67 ± 9	69 ± 9	0.4
MAP (mm Hg)[1]	76 ± 9	83 ± 9	0.05
Fasting glucose (mmol)[1]	16.6 ± 5.8	3.7 ± 0.7	<0.001
PP glucose (mmol)[1]	29.7 ± 7.5	4.3 ± 1.4	<0.001
Fasting C-peptide (pmol)[2]	0.289 (0.028–0.994)	0.485 (0.289–0.823)	<0.2
PP C-peptide (pmol)[2]	0.379 (0.053–2.424)	1.546 (0.446–2.175)	<0.001
TG (mg)[1]	108 (48–279)	85 (50–181)	<0.05
T-chol (mg)[1]	167 (96–286)	149 (113–240)	<0.08
LDL-chol (mg)[1]	112 (53–233)	92 (61–178)	<0.09
HDL-chol (mg)[1]	31 (21–61)	34 (17–55)	<0.2
ACR (mg/mmol)[2]	1.495 (0.232–4.708)	0.589 (0.371–2.600)	<0.05
SLCT (µmol Li/g/hour)[2]	0.371 (0.101–0.647)	0.340 (0.093–0.658)	<0.6

Data are expressed as [1]mean ± SD and [2]median (range)

Table 2: Clinical characteristics, glycemic index, lipid profile, ACR and SLCT of diabetics with low FCP, moderate FCP and healthy subjects

	Low FCP diabetics	Mod FCP diabetics	Control subjects
n	14	21	19
Age (years)[1]	21 ± 4	23 ± 5	23 ± 4
BMI (kg/m²)[1]	15.6 ± 2.6‡†	19.5 ± 4	19.8 ± 2.8
SBP (mm Hg)[1]	88 ± 12‡†	98 ± 7	110 ± 12
DBP (mm Hg)[1]	63 ± 8‡†	70 ± 9	69 ± 9
MAP (mm Hg)[1]	71 ± 9‡†	79 ± 7	83 ± 9
Fasting glucose (mmol/L)[1]	19.9 ± 4.7‡†	14.3 ± 5.3‡	3.7 ± 0.7
PP glucose (mmol/L)[1]	34.0 ± 5.0‡†	26.7 ± 7.5‡	4.3 ± 1.4
Fasting C-peptide (pmol/L)[2]	0.121 (0.028 – 0.160)‡§	0.527 (0.164 – 0.994)	0.485 (0.289 – 0.823)
PP C-peptide (pmol/L)[2]	0.168 (0.053 – 0.559)‡§	0.497 (0.139 – 2.424)*	1.546 (0.446 – 2.175)
TG (mg)[1]	103 (71 – 186)	112 (48 – 279)‡	85 (50 – 181)
T-chol (mg)[1]	152 (96 – 196)†	186 (105 – 286)‡	149 (113 – 240)
LDL-chol (mg)[1]	96 (58 – 137)†	132 (53 – 233)‡	92 (61 – 178)
HDL-chol (mg)[1]	33 (22 – 61)	29 (21–36)	34 (17 – 55)
ACR (mg/mmol)[2]	1.755 (0.603 – 3.914)*	1.032 (0.232 – 4.708)	0.589 (0.371 – 2.600)
SLCT (µmol Li/g/hour)[2]	0.411 (0.201 – 0.641)†	0.340 (0.101 – 0.647)	0.340 (0.093 – 0.658)

Data are expressed as [1]mean ± SD and [2]median (range), ‡$P < 0.05$ compared to healthy subjects; †$P < 0.05$ compared to moderate FCP diabetics; *$P < 0.01$ compared to healthy subjects; ‡$P < 0.0001$ compared to healthy subjects; §$P < 0.0001$ compared to moderate FCP

Table 3: Correlation-coefficient study of different parameters in diabetic subjects

	Fasting glucose	PP glucose	Fasting C-peptide	PP C-peptide
BMI	$r = -0.64$*	$r = -0.63$*	$r = 0.66$*	$r = 0.64$*
MAP	$r = -0.34$‡	$r = -0.39$‡	$r = 0.45$‡	$r = 0.54$‡
TG	NR	NR	$r = 0.39$†	$r = 0.39$†
T-chol	NR	NR	$r = 0.44$‡	$r = 0.51$‡
LDL-chol	NR	NR	$r = 0.48$‡	$r = 0.51$‡
ACR	NR	$r = 0.40$†	$r = -0.37$†	$r = -0.36$†
SLCT	NR	$r = 0.37$†	$r = -0.41$†	NR

†$P < 0.05$; ‡$P < 0.01$; *$P < 0.001$, NR: No correlation

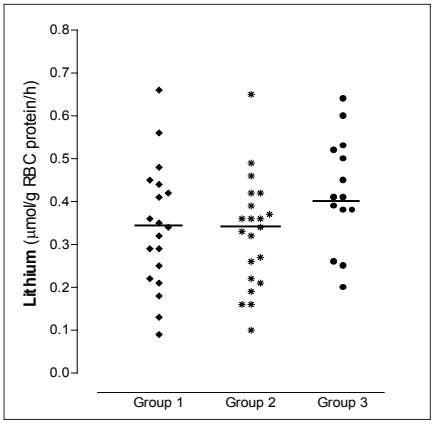

Figure 2: SLCT of healthy controls (Group 1), diabetic subjects with moderate FCP (Group 2) and low FCP (Group 3); cGroup 2 vs Group 3 (n = 19) (n = 28) (n = 7)

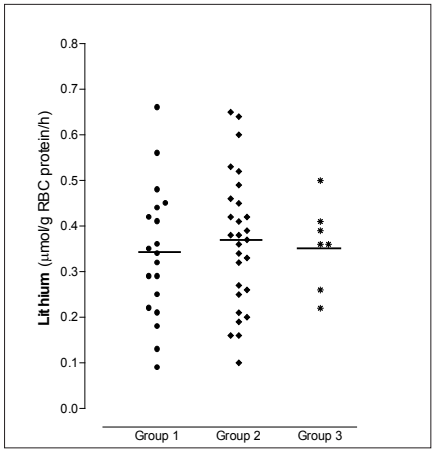

Figure 3: SLCT of healthy controls (Group 1), diabetic subjects with ACR < 2.60 (Group 2) and ACR > 2.60 (Group 3)

BMI and low blood pressure related to hypoinsulinemia and hyperglycemia found in low FCP diabetics is similar to type 1 diabetics with ketoacidosis. Hyperglycemia and hypoinsulinemia have predisposed these diabetics for elevated ACR in urine, and the presence of microalbuminuria in newly diagnosed diabetics in

this study supports previous studies on MRDM[28] and contradicts the usual finding in type 1 diabetics who develop microalbuminuria only after a couple of years.

Effect of hypertension and family history of hypertension in SLCT[7,8] are excluded in this study. Raised SLCT in type 1 diabetics with glomerular hyperfiltration,[11] microalbuminuria[12,13] and overt proteinuria[9,10] and in type 2 diabetics with microalbuminuria[15] and overt proteinuria[14] has proposed raised SLCT to be a predictor of nephropathy in these subjects. In this study, there was no significant difference in SLCT between microalbuminuric and normoalbuminuric diabetics as observed in other studies[16-18] which had concluded diabetes *per se* as the cause of elevated SLCT.

Studies in humans regarding the role of hyperglycemia *per se* suffer from limitations due to the acute need for intervention in uncontrolled diabetes or due to the development of ketosis in untreated cases. This study for the first time showed SLCT activity in untreated diabetic subjects at a severe hyperglycemic state (mean serum glucose: fasting 16.6 mmol/L, postprandial 29.7 mmol/L). No significant difference in SLCT of diabetic and non-diabetic subjects contradicts the idea that diabetes as such is accompanied by an elevated SLCT. The correlation between glycosylated hemoglobin and SLCT was also found to be absent in type 1[10] and type 2[18] diabetic subjects. But in a population-based study, positive correlation between SLCT and glycosylated hemoglobin was demonstrated.[19] Significant positive correlation (r =0.37, $P < 0.05$) of SLCT with postprandial glucose and no correlation with fasting glucose in diabetic subjects in this study indicates that extremely high degree of serum glucose may have an influence in raising the activity of the transport. It remains to be explored whether the influence is a direct consequence of hyperglycemia or it is indirectly controlled by hypoinsulinemia or some other metabolic changes related to hyperglycemia/hypoinsulinemia. Comparison of groups of these patients with severe hyperglycemia but different serum insulin levels would help to differentiate these factors.

Insulin causes the sodium retention by regulating the Na^+/H^+ antiport system.[31] SLCT activity in RBC *in vitro* experiment represents the Na^+/H^+ antiport system present in the renal tubular cells.[32] The infusion of physiologic concentration of insulin directly at the renal artery without changing the systemic concentration showed the increment of sodium retention by the respective kidney.[33] Thus, raised SLCT activity observed in studies on type 1 diabetic subjects with nephropathy[13] who are already receiving insulin, could have been due to the effect of exogenous insulin itself on SLCT. The raised SLCT in type 2 diabetic subjects with nephropathy could also be due to the direct effect of insulin as these subjects have basal hyperinsulinemia. Reports have already shown the association of increased SLCT with insulin resistance in subjects with type 1[21] and type 2[22,23] diabetes and essential hypertension.[34,35] But in all those studies on SLCT in type 1 and type 2 diabetics, the role of physiologic or subphysiologic levels of insulin on sodium retention via elevated SLCT could not be verified as there was a loss of the physiologic pulsatile insulin secretion in these subjects, and the sustained level of insulin, either exogenous or endogenous, could have variable effect directly or through other metabolic alterations. The possibility also remains that insulin does not have any direct effect on SLCT since type 1 diabetics without nephropathy do not show its elevation. The alternate possibility also remains that at low levels of insulin, the direct positive effect of the hormone on SLCT[31] is marked by some other metabolic factor(s) that may be linked with hypoinsulinemia itself. This study provided the opportunity to study the role of low levels [fasting, serum C-peptide median (range) 0.289 (0.028–0.994) pmol/L] without ketoacidosis in SLCT regulation.The study revealed significantly raised SLCT in diabetic subjects with low insulin (FCP < 0.16 pmol/L) compared to the patients with moderate FCP ($P < 0.05$). The FCP also negatively correlated with SLCT activity ($r = -0.41$, $P < 0.05$). This result is similar with that obtained in the study on hypertensive type 2 diabetic subjects.[23] So, the present data have suggested that the lower concentration in serum insulin may rather elevate SLCT activity in RBC. Such an effect of insulin, at physiologic concentration, has been reported during *in vitro* experiment.[36] Report claiming no elevation of SLCT in type 2 diabetics with endogenous and exogenous hyperinsulinemia and during *in vitro* incubation of RBC with insulin[37] and no correlation of fasting serum insulin with SLCT in type 2 diabetics[22] has also been published. The present data, therefore, suggest that insulin may have a role in the regulation of SLCT in RBCs but its effect may not be straightforward. A complex role of insulin – direct in the hyperinsulinemic range and indirect (through some humoral or metabolic events) in the hyperinsulinemic range seems to be operating on this counter transport mechanism. Studies designed *in vitro* to investigate different glucose and insulin concentrations and to investigate renal sodium handling in the present subjects with and without

nephropathy after insulin infusion can provide the greatest opportunity to correlate the effect of insulin on SLCT.

Lipid abnormalities have also been considered as a confounding variable affecting the SLCT.[20] SLCT showed positive correlation with TG, very low density lipoprotein and apolipoprotein B in type 1 diabetics,[12] with TG and T-chol in type 2 diabetics[23] and with TG in hypertensive subjects.[38] In this study, although diabetic subjects with moderate FCP had higher T-chol and LDL-chol, they showed lower SLCT compared to the low FCP group. Moreover, SLCT showed no correlation with lipids. So lipids may not influence SLCT in these subjects. However, the lipid values in these subjects were within normal limits. So, the finding may deviate considerably in hyperlipidemic range.

In conclusion, the present study suggests that raised SLCT is not a predictor of nephropathy in young diabetics of Bangladesh and the altered metabolic events in these subjects are sufficient enough to develop nephropathy. High levels of blood glucose have an influence on raising the activity of the SLCT, whereas insulin may have a complex role on SLCT with direct effect on the hyperinsulinemic range and indirect effect on the hypoinsulinemic range.

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References

1. Zatz R, Brenner BM. Pathogenesis of diabetic microangiopathy: The haemodynamic view. Am J Med 1986;80:443-53.
2. Mathiesen ER, Oxenboll B, Johansen K, Svendsen PA, Deckert T. Incipient nephropathy in type 1 (insulin-dependent) diabetes. Diabetologia 1984;26:406-10.
3. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med 1984;310:356-60.
4. Cheung CK, Cockram CS, Yeung VT, Swaminathan R. Urinary excretion of transferrin by non-insulin dependent diabetics: A marker for early complications? Clin Chem 1989;35:1672-4.
5. O'Donnell MJ, Martin P, Florkowski CM, Toop MJ, Chapman C, Holder R, *et al*. Urinary transferrin excretion in type I (insulin-dependent) diabetes mellitus. Diabet Med 1991;8:657-61.
6. Brenner BM. Hemodynamically mediated glomerular injury and the progressive nature of kidney disease. Kidney Int 1983;23:647-55.
7. Canessa M, Adragna N, Solomon HS, Connolly TM, Tosteson DC. Increased sodium-lithium countertransport in red cells of patients with essential hypertension. N Engl J Med 1980;302:772-6.
8. Woods JW, Falk RJ, Pittman AW, Klemmer PJ, Watson BS, Namboodiri K. Increased red-cell sodium-lithium countertransport in normotensive sons of hypertensive parents. N Engl J Med 1982;306:593-5.
9. Mangili R, Bending JJ, Scott G, Li LK, Gupta A, Viberti G. Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy. N Engl J Med 1988;318:146-50.
10. Krolewski AS, Canessa M, Warram JH, Laffel LM, Christlieb AR, Knowler WC, *et al*. Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. N Engl J Med 1988;318:140-5.
11. Carr S, Mbanya JC, Thomas T, Keavey P, Taylor R, Alberti KG, *et al*. Increase in glomerular filtration rate in patients with insulin-dependent diabetes and elevated erythrocyte sodium-lithium countertransport. N Engl J Med 1990;322:500-5.
12. Jones SL, Trevisan R, Tariq T, Semplicini A, Mattock M, Walker JD, *et al*. Sodium-lithium countertransport in microalbuminuric insulin-dependent diabetic patients. Hypertension 1990;15:570-5.
13. Monciotti CG, Semplicini A, Morocutti A, Maioli M, Cipollina MR, Barzon I, *et al*. Elevated sodium-lithium countertransport activity in erythrocytes is predictive of the development of microalbuminuria in IDDM. Diabetologia 1997;40:654-61.
14. Herman WH, Prior DE, Yassine MD, Weder AB. Nephropathy in NIDDM is associated with cellular marker for hypertension. Diabetes Care 1993;16:815-8.
15. Fujita J, Tsuda K, Seno M, Obayashi H, Fukui I, Seino Y. Erythrocyte sodium-lithium countertransport activity as a marker of predisposition to hypertension and diabetic nephropathy in NIDDM. Diabetes Care 1994;17:977-82.
16. Jensen S, Mathiesen ER, Norgaard K, Hommel E, Borch-Johsen K, Funder J, *et al*. Increased blood pressure and erythrocyte sodium/lithium countertransport activity are not inherited in diabetic nephropathy. Diabetologia 1990;33:619-24.
17. Crompton CH, Balfe JW, Balfe JA, Chatzilias A, Daneman D. Sodium-lithium transport in adolescents with IDDM. Relationship to incipient nephropathy and glycemic control. Diabetes Care 1994;17:704-10.
18. Gall MA, Rossing P, Jensen JS, Funder J, Parving HH. Red cell Na^+/Li^+ countertransport in non-insulin-dependent diabetics with diabetic nephropathy. Kidney Int 1991;39:135-40.
19. Trevisan M, Cirillo M, Laurenzi M. Sodium-lithium countertransport and glucose metabolism. (abstract) J Hyperten 1990;8:S124.
20. Corrocher R, Steinmayr M, Ruzzenente O, Brugnara C, Bertinato L, Mazzi M, *et al*. Elevation of red cell sodium-lithium countertransport in hyperlipidemias. Life Sci 1985;36:649-55.
21. Lopes de Faria JB, Jones SL, Macdonald F, Chambers J, Mattock MB, Viberti G. Sodium-lithium countertransport activity and insulin resistance in normotensive IDDM patients. Diabetes 1992;41:610-5.
22. Pinkney JH, Denver AE, Foyle WJ, Foster C, Yudkin JS. Insulin resistance and not hyperinsulinaemia determines erythrocyte Na+/Li+ countertransport in non-insulin-dependent diabetes mellitus. J Hum Hypertens 1995;9:685-6.
23. Giordano M, Castellino P, Solini A, Canessa ML, DeFronzo RA. Na^+/Li^+ and Na^+/H^+ countertransport activity in hypertensive non-insulin-dependent diabetic patients: Role of insulin resistance and antihypertensive treatment. Metabolism 1997;46:1316-23.
24. World Health Organisation Expert Committee on Diabetes

Mellitus: Second Report. Technical Report Series no.727. Geneva: WHO; 1985. p. 20-5.
25. Bajaj JS. Malnutrition related, ketosis-resistant diabetes mellitus–classification, causes and mechanisms. World book of diabetes in practice. In: Krall LP, editor. Amsterdem: Elsevier Science Publisher; 1986. p. 276-80.
26. Azad Khan AK, Banik NG, Mehtab H. Malnutrition-related diabetes mellitus in Bangladesh. Diabetes. In: Rifkin H, Colwell JA, Taylor SI, editors. Amsterdam: Elsevier Science Publisher; 1991. p. 944-59.
27. Bunnag SC, Chandraprasert S, Bhuripanyo, Lueprasitasakul W. Clinical features of malnutrition–related diabetes mellitus. Diabetes. In: Baba S, Kaneko T, editors. Amsterdam: Elsevier Science Publisher. 1994. p. 258-62.
28. Azad Khan AK, Ali L. Tropical calcific pancreatitis and fibrocalculus pancreatic diabetes in Bangladesh. J Gastroenterol Hepatol 1997;12:S48-52.
29. Ragoobirsingh D, Bennett F, Morrisos EY. Kidney function in phasic insulin dependent diabetes mellitus in Jamaica. West Indian Med J 1997;46:22-4.
30. Report of the Expert Committee on the Diagonsis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.
31. Moore RD. Stimulation of Na: H exchange by insulin. Biophys J 1981;33:203-10.
32. Mahnensmith RL, Aronson PS. The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological processes. Circ Res 1985;56:773-88.
33. DeFronzo RA, Goldberg M, Agus ZS. The effects of glucose and insulin on renal electrolyte transport. J Clin Invest 1976;58:83-90.
34. Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, *et al*. Insulin resistance in essential hypertension. N Engl J Med 1987;317:350-7.
35. Doria A, Fioretto P, Avogaro A, Carraro A, Morocutti A, Trevisan R, *et al*. Insulin resistance is associated with high sodium-lithium countertransport in essential hypertension. Am J Physiol 1991;261:E684-91.
36. Foyle WJ, Drury PL. Insulin reduces lithium-sodium counter-transport *in vitro* (abstract). Diabetic Med 1989;6:2A.
37. Senda T, Serizawa N, Negishi K, Katayama S. Elevated erythrocyte sodium-lithium counter-transport in hypertensive patients with non-insulin-dependent diabetes mellitus. Diabetes Res Clin Pract 1996;31:37-44.
38. Strazzullo P, Cappuccio FP, Trevisan M, Siani A, Barba G, Ragone E, *et al*. The relationship of erythrocyte sodium-lithium countertransport to blood pressure and metabolic abnormalities in a sample of untreated middle-aged male workers. J Hypertens 1993;11:815-22

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